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Seroprevalence and Anti-HEV Persistence in the General Population of the Republic of San Marino

Maria Rapicetta,[1]* Loreta Anesti Kondili,[1] Stefano Pretolani,[2] Tommaso Stroffolini,[3] Paola Chionne,[1] Umbertina Villano,[1] Elisabetta Madonna,[1] Ferruccio Casali,[4] and Giovanni Gasbarrini[5]

[1]Laboratory of Virology, Istituto Superiore di Sanità, Rome, Italy
[2]Urgent Medicine, General Hospital S. Orsola of Bologna, Bologna, Italy
[3]Department of Hepatology, Hospital S. Giacomo, Rome, Italy
[4]Hospital State, Republic of San Marino, Italy
[5]Internal Medicine II, Hospital Gemelli, Rome, Italy

The prevalence of anti-HEV was assessed in 2,233 subjects aged 20–79 years in the Republic of San Marino in the years 1990–1991. The sera were tested by ELISA and further confirmed by Western blot (WB) analysis. The overall anti-HEV prevalence was 1.5%. A significant trend by age was observed. Anti-HEV prevalence was 0.6% in subjects <30 years and 3.3% in those older than 70 years of age. Family size larger than four persons (OR = 3.8; 95% CI = 1.8–13.2) was the sole independent predictor of anti-HEV positivity in the multivariate analysis. Anti-HAV and anti-HEV prevalences did not show a parallel trend by age. No association was found either between hepatitis E virus (HEV) or hepatitis C virus (HCV) infections. Follow-up samples 5 years apart were available for 38 out of 54 (70%) anti-HEV ELISA-positive subjects. Eight out of 22 (37%) WB-confirmed anti-HEV–positive subjects were still anti-HEV–positive after 5 years. However, anti-HEV remained positive in all but two (75%) of the subjects with WB-confirmed ELISA positivity value of S/CO $\geq$ 2 (cutoff 1.2), but in only 2 out of the 14 subjects (14%) with a WB-confirmed ELISA positivity value of S/CO < 2 (P < 0.005). None of the 16 subjects ELISA-positive but not WB-confirmed was anti-HEV–positive 5 years apart. Therefore, only a relative proportion of subjects once infected with HEV maintain for at least 5 years anti-HEV antibodies. ***J. Med. Virol. 58:49–53, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: hepatitis E; HEV antibody; prevalence; Western blot

INTRODUCTION

Hepatitis E virus (HEV) is a single-stranded positive-sense RNA virus. HEV infection is usually a self-limiting illness that lasts 3–4 weeks with an incubation period ranging from 2 to 9 weeks. The establishment of chronic HEV infection has not been described. Hepatitis caused by HEV has been reported in both endemic and epidemic forms in Asia, Africa, and Mexico [Koziel, 1996]. The epidemics generally occur in developing countries and are mainly the result of ingestion of water contaminated by the virus [Ramalingaswami and Purcell, 1988; Shidrawi et al., 1994]. In areas in which HEV infection is not endemic, HEV infection cases have been reported mainly among immigrants or travelers returning from HEV endemic regions [DeCock et al., 1987; Center for Disease Control and Prevention, 1993]. In several of these non-HEV endemic areas, a few sporadic clinical cases and positivity for anti-HEV have been reported among persons who have not traveled to endemic areas. The mode of HEV transmission in these cases has not been determined [Toursaget et al., 1994]. It has been suggested that hepatitis E virus may also be transmitted by parenteral routes [Psichogiou et al., 1995; Al-Fawaz et al., 1996; Gessoni and Manoni, 1996]. Data on seroprevalence of anti-HEV in developed countries suggest the possibility that the virus may spread in a manner different from that previously recognized (mainly fecal-oral). The possible existence of vector reservoirs that maintain the virus or related viruses in the environment has also been suggested [Mushahwar et al., 1996].

Anti-HEV seroprevalence of 1%–5% in blood donors has been reported from several countries where HEV is not endemic using commercially available recombinant protein-based tests. HEV prevalence has recently been described in northeastern and central Italy [Zanetti et al., 1994; Gessoni and Manoni, 1996; Stroffolini et al., 1996].

The aim of this study was to assess anti-HEV preva-

*Correspondence to: Dr. Maria Rapicetta, Laboratory of Virology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.

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lence in a general adult population in the Republic of San Marino in north-central Italy. The effect, if any, of sociodemographic factors on anti-HEV prevalence has also been evaluated. Finally, the persistence of such antibodies 5 years apart has been assessed.

MATERIALS AND METHODS

Study Population

The Republic of San Marino (25,000 inhabitants) is located in north-central Italy. The population is very stable because of strict immigration laws as well as economic incentives for the residents. Since 1960, a dramatic improvement of economic and sanitary conditions in this area has been made, as reflected by the reports of the Annual Demographics and Economic Statistics of the Republic of San Marino.

The study population included all subjects 20–80 years old (17,000) residing in San Marino in January 1990. A random stratified sampling procedure with proportional allocation by age, sex, and district of residence was used. The selected subjects were invited by letter or phone call for an interview and blood tests. All subjects participating in the study were interviewed by the same physician using a standard questionnaire containing information on age, sex, family size, and level of education. The study period was June 1990–July 1991. The survey was approved by the ethic committee of the Istituto per la Sicurezza Sociale of the Republic of San Marino.

Serological Assays

Serum samples were tested for specific IgG antibodies directed against HEV by a commercial enzyme immunoassay (Abbott HEV EIA), which uses recombinant antigens from the open reading frames (ORFs) 2 and 3 of the HEV genome. The solid phase is coated with the two different antigens that were expressed in *E. coli* with a fusion protein (CKS). The antigens were constituted respectively by all the 123 amino acids from the ORF3-encoded protein (8-5) and 327 amino acids from the C terminus of the ORF2-encoded protein (SG-3). The reaction is revealed by a human anti-IgG conjugated with horseradish peroxidase.

For determining anti-HEV antibody, the following procedure was applied: all the initially reactive ELISA samples were retested and only those repeatedly reactive by EIA (cutoff ratio ≥ 1.2) were further tested by a Western blot (WB) assay (Abbott, Wiesbaden, Germany) that was used as a confirmatory assay. This test included the same two antigens used in the EIA blotted on a nitrocellulose strip. CKS protein was added separately. A sample was considered "confirmed positive" when it reacted against at least one recombinant HEV antigen, but not against CKS.

All sera were tested for the presence of anti-HCV by third-generation ELISA (Ortho) and by third-generation RIBA (Ortho) supplemental assay. All sera were tested by enzyme-linked immunosorbent assay (ELISA) for anti-HAV (Abbott Laboratories, North Chicago, IL).

TABLE 1. Age-Specific Prevalence of anti-HEV in San Marino[a]

Age-groups, years	HEV, number positive/number studied	%
<30	3/517	0.6
30–39	3/448	0.7
40–49	6/430	1.4
50–59	9/366	2.5
60–69	6/292	2.1
>70	6/180	3.3
Total	33/2,233	1.5

[a]Chi-square for linear trend $P < 0.05$.

Statistical Analysis

Differences in proportions were evaluated by a chi-square test and a Fisher exact test. A P value of <0.05 was considered to be significant. The crude odds ratios (OR) for the association linking HEV infection to sociodemographic characteristics of the subjects were evaluated by univariate analysis. The independent effect of the sociodemographic characteristics on anti-HEV positivity was evaluated by a multiple logistic regression analysis. ORs were evaluated using as a reference the category with the most favorable level of exposure (i.e., youngest age, lowest family size, and highest number of years of education).

RESULTS

Over 3/4 (77%) of the sampled population agreed to participate in the study. Nonresponders were equally distributed in all age groups; they did not differ from responders in respect to sociodemographic characteristics. The studied sample consisted of 2,233 subjects.

Table I shows the age-specific prevalence of anti-HEV. Out of the 2,233 subjects studied, 54 were initially repeatedly reactive by ELISA; only 33 were reactive by WB. Thus, the overall WB-confirmed anti-HEV prevalence was 1.5%. A significant increasing trend by age (chi-square for linear trend $P < 0.05$) can be observed. The prevalence increased from 0.6% in subjects younger than 30 years of age to 3.3% in those older than 70 years of age.

Table II reports the frequency of HEV seropositivity by sociodemographic characteristics of the subjects. Age greater than 50 years (OR 3.4; CI 95% = 1.6–7.5) and family size larger than four persons (OR 3.7; CI 95% = 1.1–12.6) were found to be associated to HEV exposure at the univariate analysis. Sex and years of education produced results that are both unassociated to HEV exposure.

When each variable is adjusted for the confounding effect of all other variables by multiple logistic analysis, we observed that family size larger than four persons was the sole factor independently associated to the presence of anti-HEV (OR 3.8; CI 95% = 1.8–13.2).

The prevalence of anti-HCV and anti-HAV in this population was 1.3% and 74.2%, respectively. None of the anti-HCV–positive subjects was anti-HEV–positive. The age-specific anti-HAV and anti-HEV prevalences (Table III) do not show a parallel trend by

TABLE II. Prevalence of Anti-HEV by Sociodemographic Factors in San Marino[a]

Characteristics	Number positive/number tested	%	OR crude (CI 95%)	OR adjusted[b] (CI 95%)
Age				
<50	11/1,395	0.8	1	
⩾50	22/838	2.5	3.4 (1.6–7.5)	4.5 (0.9–22.3)
Sex				
M	14/1,046	1.3	1	
F	19/1,187	1.6	1.2 (0.6–2.5)	2.5 (0.6–9.7)
N subject in a household				
⩽4	8/497	1.6	1	
>4	4/70	5.7	3.7 (1.1–12.6)	3.8 (1.8–13.2)
Years of schooling				
>13	6/260	2	1	
9/13	3/245	1.2	0.5 (0.1–2.4)	Not computable
⩽8	11/410	2.7	1.2 (0.4–3.6)	Not computable

[a]Crude and adjusted odds ratio (OR) derived by multiple logistic regression analysis.
[b]Each variable is adjusted for the confounding effect of all other listed in the table.

TABLE III. Anti-HEV Positivity According to Anti-HAV Status[a]

Age	Anti-HAV$^+$ and anti-HEV$^+$	%	Anti-HAV$^-$ and anti-HEV$^+$	%
<30	1/148	0.6	2/369	0.5
31–40	0/294	0	3/154	1.9
41–50	5/391	1.2	1/39	2.5
51–60	9/364	2.4	0/2	0
61–70	6/287	2	0/5	0
>70	6/174	3.4	0/3	0
Total	27/1,658	1.6	6/572	1

[a]In three subjects anti-HAV was not tested.

TABLE IV. Antibody Persistence 5 Years Apart in Anti-HEV–Positive Subjects

Subjects (n = 38)	Number tested	Mean age	Anti-HEV positivity (5 years apart)	%
Anti-HEV ELISA-positive S/CO ⩾ 2 WB-confirmed	8	58 ± 6	6	75
Anti-HEV ELISA-positive S/CO < 2 WB-confirmed	14	49 ± 4	2	14
Anti-HEV ELISA-positive WB-negative	16	55 ± 17	0	0
Total	38	55 ± 14	8	21

age. Anti-HEV positivity was observed in 1.6% (27/1,658) of the anti-HAV–positive subjects and in 1% (6/572) of those anti-HAV–negative (OR 1.6; CI 95% = 0.6–4.3). The lack of association between HAV and HEV infections was still present after adjustment for the confounding effects of other factors (age, sex, years of education, and family size) (OR 3.6; CI 95% = 0.8–15.9). As shown in Table III, an inverse trend of anti-HEV positivity by age was observed comparing anti-HAV–positive and anti-HAV–negative subjects.

Follow-up samples 5 years apart were available for 38 out of the 54 (70%) anti-HEV ELISA-positive subjects (Table IV). Eight out of 22 (37%) WB-confirmed anti-HEV–positive subjects were still anti-HEV–positive 5 years apart. However, anti-HEV IgG remained positive in all but 2 (75%) of the subjects with WB-confirmed ELISA positivity value of S/CO ⩾ 2 (CO 1.2), and in only 2 out of the 14 subjects (14%) with a WB-confirmed ELISA positivity value of S/CO < 2 ($P < 0.05$). Mean age of the above subjects with confirmed and unconfirmed WB was 58 ± 6 years and 49 ± 4 years, respectively. None of the 16 subjects ELISA-positive but not WB-confirmed was anti-HEV–positive 5 years apart (see Table IV).

DISCUSSION

This study, carried out in a large random sample of a general population, indicates an overall prevalence rate of 1.5%, which is in agreement with several data from other surveys [Pujol et al., 1994; Bernal et al., 1996]. In northwestern Italy, anti-HEV seroprevalences of 0.74 [Zanetti et al., 1994] and 2.6 [Gessoni and Manoni, 1996] were reported in healthy subjects. In a central Italian town, the anti-HEV prevalence was 2.6% in an open population [Stroffolini et al., 1996].

The relative discrepancies are present possibly due to the use of different assays and modalities of use.

In this study, to avoid false positive results, a confirmatory WB assay was applied to all samples repeatedly anti-HEV ELISA-positive with a cutoff value ≥1.2. The anti-HEV prevalence, assessed only by ELISA, was 2.7%; it decreased to 1.5% according to WB results.

The observed age prevalence is consistent with a cohort effect, i.e., decreased risk of infection along generations as a consequence of improved sanitary and socioeconomic conditions over time. The low prevalence value in subjects 20–30 years old, in this nonendemic area, may suggest a possible person-to-person transmission, as reported in other studies [Khuroo and Dar, 1992; Gessoni and Manoni, 1996]. This is supported by the association found with a larger family size (more than four persons).

A marked trend of seroreactivity associated with increasing age was also observed in other studies among persons living in HEV endemic-nonendemic regions [Pujol et al., 1994; Arankalle et al., 1995; Bernal et al., 1996; Stroffolini et al., 1996]. In these studies, the possibility of a decreased spread of infection due to remarkable improvements in socioeconomic conditions was considered; in addition, the possibility that HEV somehow has a selective tropism for the liver cells of adults was also suggested [Balayan, 1987].

Despite high rates of anti-HAV positivity present in all age groups, no association was found between HAV and HEV infections. The seroprevalence of more than 90% for anti-HAV in the oldest age reflects the stability of detectable antibodies for anti-HAV infections [Stroffolini et al., 1997]. In contrast, persistence of HEV seropositivity during lifetime is not well documented.

It has been reported that the persistence of HEV IgG is relatively short (1–1.5 years) after an acute infection [Koshy et al., 1996]. A persistence of anti-HEV positivity for at least 3.5 years in a U.S. traveler to an endemic country has been reported [Dawson et al., 1992]. In our study, detectable HEV IgG was positive 5 years apart in the majority of cases with an anti-HEV ELISA S/CO value ≥2, suggesting a long-lasting antibodies persistence in subjects with specific characteristics.

The persistence of anti-HEV 5 years apart was more likely in subjects older than 50 years of age in comparison to those younger than 30 years of age. It has been suggested that HEV sporadic infection in children, adolescents, or young adults has generally a clinical or subclinical course followed by gradual loss of immunity [Zakaria et al., 1988; Hyams et al., 1991; Goldsmith et al., 1992; Mushahwar et al., 1996; Balayan, 1997]. In these cases, IgG could be present at low titers and consequently may last briefly. In contrast, it might be hypothesized that adults or older subjects, once infected, can develop higher titers of IgG lasting more than 5 years. Different routes of transmission may also be postulated. In children, the infection could be more frequently transmitted enterically. In adults, other routes of transmission could not be excluded [Psichogiou et al., 1995; Al-Fawaz et al., 1996]. However, the kinetics of anti-HEV IgG after the acute phase or in cases of sporadically acquired HEV infection are not completely clear. The likelihood of infection caused by strains of HEV with different pathogenetic characteristics or the presence of cross-reacting antibodies cannot be ruled out [Mushahwar et al., 1996].

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